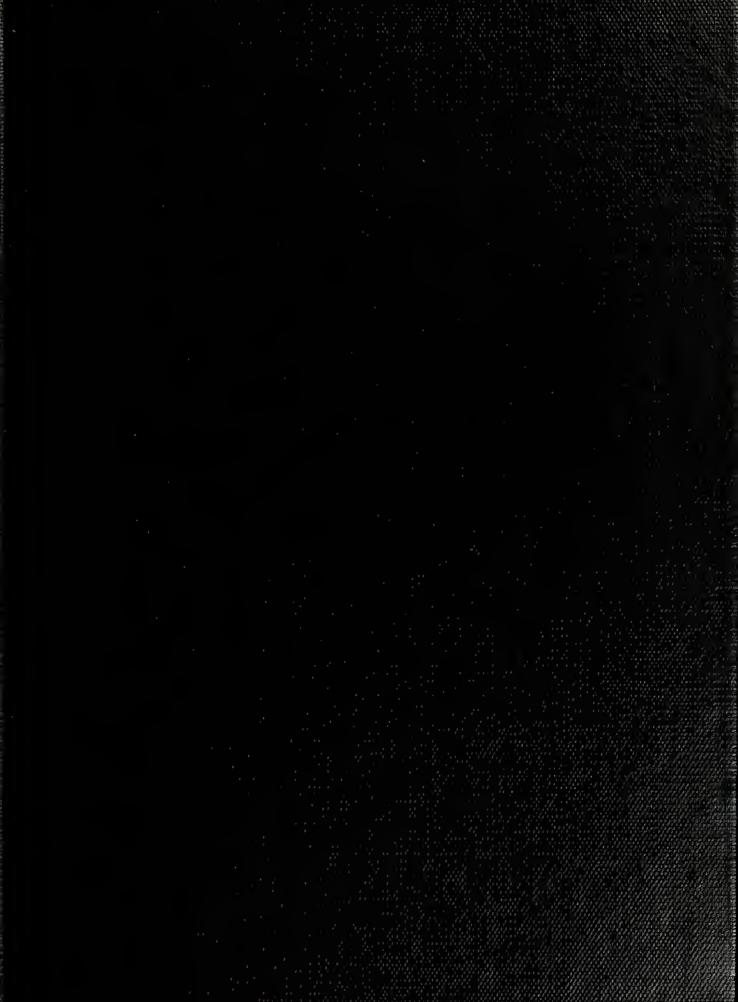
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Of CONE COLLECTION on Douglas-fir Seed Processing and Germination:



Donald L.Olson Roy R.Silen

Abstract

Cones were harvested between August 12 and September 15, 1966, from 309 parent trees. Seed weights and germination rose sharply from August 12 to 25 and slowly thereafter. The early-picked lots required inordinate time in processing and special germination techniques and produced very ragged bed densities. Correlation between laboratory and nursery germination was 0.83, with field germination generally higher. Fully ripened cones picked in 1968 from the early-collected, low-germination trees of 1966 germinated normally.

KEYWORDS: Cone collection, germination (seed), seed weights.



The first implementation of the Progressive Tree Improvement Programwas by the Crown Zellerbach Corporation²/ at Vernonia, Oregon. Early phases of the program involved cone collection in 1966 from 309 individual parent Douglas-fir trees over a 35-day period and the growing of the seedling families in 1967 and 1968 in our experimental nursery at Corvallis, Oregon The large number of parent trees provided us an excellent opportunity to document and evaluate all cone collection, seed processing, and nursery steps, the results of which are reported in this paper. Of all the factors that contributed to variation, seed immaturity arising from collecting cones too early has proved to contribute by far the most problems. It increased time required to process and use the seed and was a major contributor to variation in nursery bed density.

Cone Collection and Seed Handling

The 309 parent trees were located in 35-year-old stands of the Oregon Coast Ranges along roads of the 80,000-acre Ed Stamm Tree Farm of the Crown Zellerbach Corporation surrounding Vernonia, Oregon. Elevations range from about 400 to 1,700 feet (130 to 550 meters). All bore one-half bushel (15 liters) or more of cones, resulting in a total collection of about 75 pounds (35 kilograms) of seed.

A half bushel or more of cones was collected from each tree for opera-

1/ Roy R. Silen. A simple, progressive, tree improvement program for Douglas-fir. USDA Forest Service Research Note PNW-45, 13 p. Pacific Northwest Forest and Range Experiment Station, Portland, Oreg., 1966.

tional seedling production. A 1/4-bushel (2 gallon) sample was also collected for growing progeny test seedlings at Corvallis. The results of this paper pertain only to the 1/4-bushel samples, which averaged about 200 cones.

At cone-picking time, there was the serious problem of completing such a large collection in a short period of time. Because of the limited number of cone pickers, it was necessary to start picking as early as practical in order to finish before seedfall. Cone maturity was determined regularly from cone and embryo inspections from August 1 onward. Based upon embryo length and firmness of gametophytic tissue, cones were judged (inaccurately) to be mature enough on August 12 to start low elevation collections. Cone picking continued at a steady rate of about nine trees per working day which included most Saturdays and Sundays until the job was finished September 15. Cones were collected in numerical order starting with tree No. 1 and ending with tree No. 310 (with one tree skipped because squirrels had preempted the harvest). Hence, knowing the daily rate of collection, the actual date of collection for any tree could be determined with accuracy of a day or two. Even with this early start, the last collections were made in trees which were beginning to shed seed.

Cones were handled with more than the usual care in the field. The cones were stored briefly in large paper bags on a cement-floored garage in Vernonia. In order to assure good ventilation, at no time was one bag allowed to contact another bag. The cones were transported to Camas, Washington, whenever a pickup load had accumulated. There they were spread out on benches in a heated greenhouse for rapid drying. Even with such careful attention, many of the cones developed some mold. We had intended to prevent molding by getting cones rapidly into drying conditions, but handling so many parental lots overwhelmed the drying facilities and molds became a small but acceptable problem.

^{2/} Crown Zellerbach Corporation funded collection of data on parent trees, seed processing, and laboratory germination testing for the study. Special acknowledgment is given their forester, Richard Robertson, who made the special collection of seed in 1968 providing proof that low-germinating lots in original collection were normal in germination when collected in late September.

Some molding is practically unavoidable even in well-planned commercial collections during heavy seed years. When finally dry in November, the cones from the 309 1/4-bushel samples were transported to Corvallis. Seed processing was completed by late December. The entire cone handling procedure is comparable to conditions encountered with careful operational practice.

In order to maximize seed yield, the 1/4-bushel lots were processed with laboratory scale equipment. For tumbling, we used a 24-inch (0.6-meter) screen tumber rotating at about 32 revolutions per minute. Seeds were then cleaned in a small, commercial air-screen seed cleaner. Seeds from this cleaner were given a final cleaning with desk top cleaner. Most lots were cleaned to practically 100-percentfull seed, but some lots dropped to 95-percent.

Complete records for each lot were made during cone and seed processing. Cones per bushel, seeds per cone, seed yield, trash content, and seed weight are summarized in table 1. In addi-

tion, the amount of mold, insect damage, and the extraction difficulty were rated (none, moderate, or severe). Percents by class are given in table 2.

Early in the processing it became obvious that seed yields in over one-quarter of the lots were poor because cones were not opening fully, or because the seeds were clinging tenaciously to the cone scales. A tabula- . tion was made of causes for poor yield on the 75 poorest lots. In 36 lots (48 percent), seed was retained in the cone because scales failed to spread due to coneborer damage or mold. Of the 39 lots in which scales opened, seed was retained on the scales by midge in 16 lots (21 percent), by mold on 16 lots (21 percent), and by both insects and mold in 7 lots (9 percent). To increase seed yield, 32 of the 75 lots were soaked, dried, and retumbled. Seed yield from soaked cones was often greater than the initial yield.

Seed Weights

The problem with early-picked cones was not detected until weights per 100 seed were calculated. Seventy-six percent of the lots collected before August 22 weighed less than

Table 1.--Summary of data on cones and seeds from 309 parent trees at Vernonia, Oregon, 1966, covering collections, processing, and $ext{continuition}^{1/2}$

Measure	Number of cones per bushel	Cone cut index2/	Seed weight (grams per 100 seeds)	Trash (grams per 100 seeds)	Seeds per cone	Percent germination 3/	
						In vitro	Nursery
Range	344-1,784	0.8-10.5	0.549-1.437	0.005-1.486	3.9-53.7	0-94.0	1.0-100.0
Mean	775.62	5.631	1.005	.094	25.588	44.10	52.78
Standard deviation	196	1.855	.185	.127	9.318	27.48	28.43
Coefficient of variation	25.3	32.9	18.4	<u>4/</u> 135.1	36.4	62.3	53.9

 $[\]frac{1}{2}$ A table of basic data on 309 trees is available on request to authors.

^{3/} Roy R. Silen. A laboratory seed separator. Forest Science 10(2): 222-223.

 $[\]frac{2}{}$ Number of filled seeds counted on face of longitudinally cut cone.

 $[\]frac{3}{2}$ Oregon State University Seed Laboratory. Four lots of 50 seed. Does not include abnormal seedlings.

^{4/} Distribution is highly skewed.

Table 2.--Percentage of lots by severity class with problems from mold and insects, and with seed extraction difficulties

Mold	Insects	Extraction difficulty
17	17	26
63	76	46
20	7	28
	17 63	17 17 63 76

0.90 gram per 100 seed. Lots collected after August 22 averaged 1.1 grams per 100 seed, and only 14 percent weighed less than 0.90 gram per 100 seed. The cone lots that failed

to open, or had appreciable mold, were found to be primarily from early-picked lots. In reviewing cone and seed processing time records, we found that these lots had required 3 to 10 times as much processing effort per unit weight of seed as the fully mature lots. They were tumbled longer, yielded less, and often required soaking, redrying, and more tumbling. Some cones had to be broken open by hand.

In figure 1, seed weight is plotted against collection date. An average weight for August 12 collections was 0.85 gram per 100 seed. For 10 days, seed weights were very low; then they climbed to about 1.05 grams, about a 20-percent rise for those seed collected about August 25, and rose gradually to average above 1.10 grams for collections

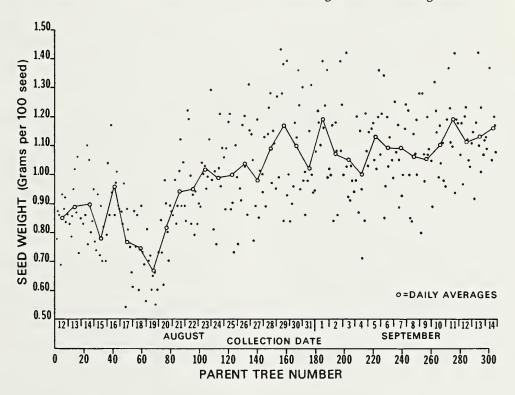


Figure 1.--Weight distribution of 100-seed collections for 309 parent trees by parent tree number and date of cone collection. Line connects daily seed-weight averages. Data show cones picked before August 26 as lighter than after this date. Distinctly lighter seed is seen for lots 40 to 75 collected about August 17-20, which originated 300 to 400 feet (90 to 120 meters) higher than trees picked earlier or later.

after September 1. But figure 1 also shows a very obvious dip to below 0.70 gram in the average weight in seed lots 40 to 75. The earliest collections in August were begun at about 1,000-foot (300-meter) elevation (lots 1 to 39), then the crew was moved to a 1,300-foot (400-meter) elevation (lots 40 to 75), and then back down to about 900-foot (275meter) elevation for further collections. We hypothesized that this higher elevation collection was sufficiently less mature to be reflected in the seed weight. Subsequent observations of these immature lots revealed that they constituted the main source of serious problems with laboratory and field germination.

Seed Weight-Yield Relationship

A regression analysis based on 309 seed lots summarized in table 1 confirmed suspected relationships. Seed weight increased linearly with collection date (r = +0.53) and size of cone (r = +0.50). Number of seeds per bushel of cones increased with number of seeds per cone (r = +0.80)and number of cones per bushel (r = +0.29) and decreased as more extraneous matter was included in the processed seed (r = -0.42). The latter relationship resulted because poor-yielding lots were tumbled longer and, as a result, produced more broken bracts and small chips of stems and scales. As expected, number of cones per bushel dropped with increasing numbers of seeds per cone (r = -0.25). Best seed yields were from fully ripened cones having a high number of seeds per cone, or from lots with lower seed numbers per cone but with more cones per bushel. No unexpected relationships developed, but the generally low correlation coefficients from so large a sample give a realistic indication of the variation in a single operational collection.

Cutting Test Count vs. Seeds Per Cone

From each 1/4-bushel sample of undried cones, 10 cones were cut longitudinally for a record of the number of full seed seen on one cut surface. This is a standard cone testing procedure. A regression, $\hat{Y} = 4.61 + 3.73X$, expresses the relationship of this average seed count and actual seed yield per cone after processing for a parent tree (fig. 2). A rule-of-thumb using a multiplier of 4.5 (mean seed per cone divided by mean cut seed in table 1) for each cut seed gives a reasonably accurate yield estimate over most of the range of our data. Similarly, a multiplier of 3,500 per cut seed provides a rough estimate of number of seeds per bushel of cones.

Such multipliers, or regressions, could be greatly in error for single lots. For example, a cut cone count of 5 seed embraces a range of filled seed per cone of 10 to 40. Besides normal variation, this reflects extraction difficulties caused by mold, insects, and early collection. The relationships of cut test to average seed per cone (r = +0.74) or to total seed in a lot (r = +0.62) and weight of extraneous material (r = -0.31), though highly significant, leave half or more of the variation in each unexplained.

Laboratory Germination

All the lots were tested for germination by the Oregon State University Seed Laboratory. Only 200 seed from each lot were available for germination tests because many lots had low seed yield. A germination test with no prechill treatment $\frac{4}{}$ was recommended by the laboratory for the freshly harvested seed. The 200 seed were

^{4/} Western Forest Tree Seed Council. Sampling and service testing, western conifer seeds. 1966.

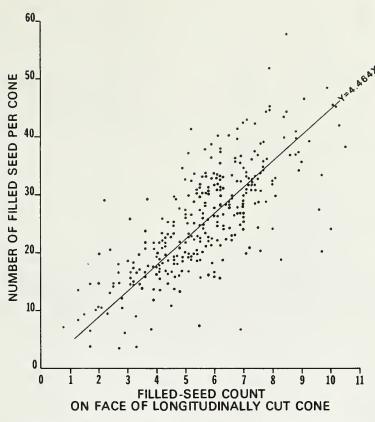


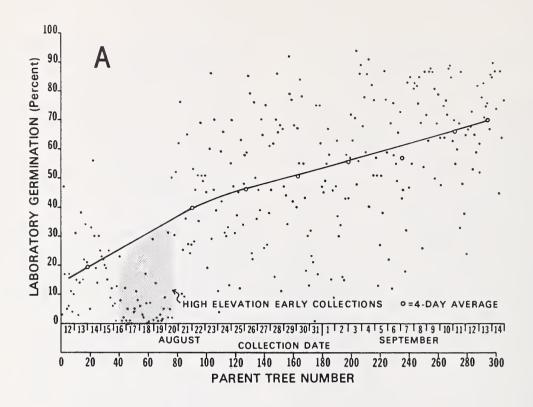
Figure 2.--Relationship of seed yield to filled seed count on face of a longitudinally cut cone.

divided between four germination dishes with 50 seed per dish. Summary of laboratory germination test means are also shown in table 1.

Germination figures from the OSU Seed Laboratory plotted against time of collection most vividly reveal the losses in early-picked cones (fig. 3A). In general, lots that were lightest in the early-picked group dropped in germination percent in an even more pronounced way than would be suspected from their lighter seed weights. Practically all the seed lots below 20 percent in laboratory germination were from parent trees 1-90. Only a few of the lots from parent trees 40 to 75, collected at 1,300-foot (400-meter) elevation, were above 10-percent germination.

Nursery Germination

Effects of immature-seed collection were even more evident in the seedbed. The intent was to sow all the seed in our nursery beds at Corvallis. When estimates of expected seedling yields became available from laboratory germination test results, not enough filled seed remained in 40 of the 1/4-bushel lots to produce the 150 seedlings desired for progeny tests. We decided the maximum number of seedlings would be best obtained if the 40 lots (34 of the 40 being among parents 1 to 90) were germinated in the laboratory and then planted in the beds as germinants. When we germinated this seed in a perlite medium, we had another disaster. Bacteria killed the growing points of emerging hypocotyls. Despite the use of Phygon on the seed after stratification and Terramycin



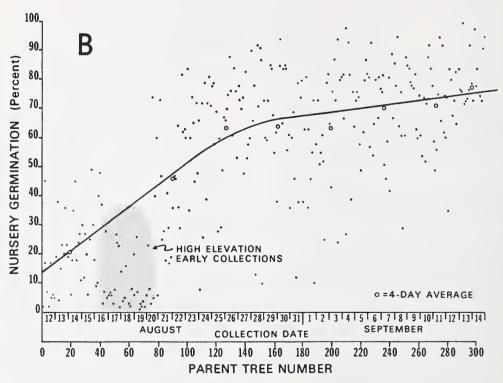


Figure 3.--Laboratory (A) and field (B) germination percent of seed from 309 parents by collection date. Freehand curves of average germination exclude data of parent numbers 40 to 75 in the shaded area.

in the perlite, seedling losses were severe. Further unsuccessful germination attempts in other media resulted in loss of much of the badly needed seed. We finally found that a germination medium of pure peat moss would completely prevent bacteria losses, producing healthy germinants, but not in time to assure 150 germinants from seed remaining in 8 of the 309 lots.

Seed of the remaining 269 lots having ample seed (309 minus 40) were sown in nursery beds. Laboratory germination test results were used to adjust the amount of seed sown per 4-foot row to produce a row of about 40 plants. An estimate of nursery germination was made in June by counting seedlings that appeared above soil surface. Such an estimate is conservative, since seeds that germinate but do not emerge above the surface are not included. A high variation was observed between laboratory and field germination. Some lots with laboratory germination estimated below 30 percent had excellent field germination to produce overstocked rows. Others, including some with high estimates of laboratory germination percent, germinated poorly in the beds to give poorly stocked rows.

Percent of nursery germination is displayed by collection date in figure 3B. Germination of the 40 lots given special treatment is also included. In figure 3 poorly germinating seeds from higher elevation (parents 40-75) are shaded to remove them from the remaining data. Doing so results in a smooth curve of average germination over time. The nursery germination curve is the more readily interpreted in terms of seed ripening. Germination percent rose rapidly from about 15 percent for August 12 seed to 60 percent for August 26 seed. Enough lots were still requiring additional ripening after August 26 to provide for another 15-percent rise by September 15 when seedfall began. Laboratory germination showed greater

variation and a more gradual rise through the entire period. Since laboratory germination on fresh seed without stratification was consistently lower, these lots may have benefited from the stratification used before nursery sowing. Even so, laboratory and field germination were not well correlated, as seen in figure 4. The coefficient of correlation r is 0.83, indicating only 69 percent of the variation explained by the relationship.

Figure 5 shows most dramatically the overall effects of the early-picked lots on nursery bed stocking. It is a vertical mosaic of two portions of one replication of our seedbeds at Corvallis. Each row has seedlings from a single parent. We purposely sowed this one replication in the order that cones were collected, i.e., in consecutive numerical order from parent trees 1 to 310. Rows 91 to 309 from parent trees collected later than August 26 (partly shown in figure 5B) were quite uniform in germination. In contrast, rows for parent trees 1 to 90, which were collected from August 12 to August 26, are very spotty in germination (figure 5A). These rows had known low germination, so most were sown with over 600 filled seed per row. Many more and even larger gaps would have shown in the photograph had not 34 seed lots with lower germination been grown elsewhere with special treatment.

Average field germination of the fully ripe lots (parent trees 150 to 300) was nearly 70 percent. In this group, 88 percent of all the lots germinated above 50 percent. As a practical conclusion, it is apparent that any lot of fully ripe seed comprised of several parent trees, handled well and uniformly sown, should have eliminated most problems leading to uneven seedbed densities. On an individual parent basis, our bed densities in lots 150 to 300 benefited somewhat because we adjusted sowing rates to reflect laboratory germination differences rather than sowing the same number of seed per row.

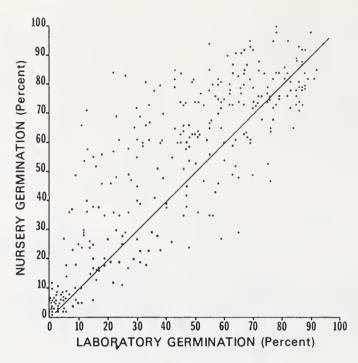
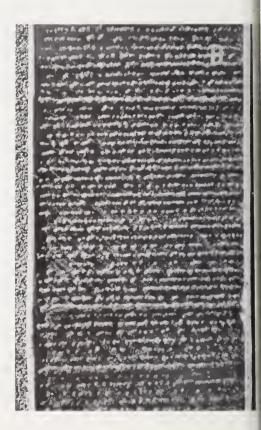


Figure 4.--Field versus laboratory germination percent. Line shows expected 1:1 relationship. Field germination was generally better than predicted from laboratory germination.



Figure 5.--Low seedbed densities from earlypicked (A) cones contrasted with high densities from fully-ripened cones (B). Each row in (A) represents a single parent's seed from collections made between August 12 and August 20. (B) represents seed collections between September 5 and September 10. Overly dense rows result from dense sowing of seed thought to have poor germinability based upon laboratory germination test.



Retrial With Fully Ripened Seed

Evidence was sought that parent trees showing low germination from early collections in 1966 were otherwise normal. In September 1968, during another good seed year, cones were collected from 13 parent trees. All 13 were from the group picked before August 25 in 1966, which had averaged only 6-percent nursery germination that year. A new nursery germination count on 1968 seed based upon three rows of 200 seeds per parent averaged a normal 72 percent (table 3), a figure corresponding well to the germination percent of fully ripened seed in 1966 (fig. 3B).

Table 3.--Comparison of nursery germination of 1966 and 1968 seed collected from 13 of the parent trees showing low 1966 germination. The 72-percent average germination in 1968 is evidence that low germination of 1966 was due to early collection

Parent	Nursery germination percent				
number	1968 seed <u>1</u> /	1966 seed ² /			
1	74	2			
5	22	2			
17	86	23			
27	45	11			
42	81	3			
49	99	3			
61	61	3			
63	98	20			
67	98	1			
69	88	2			
71	58	2			
74	82	8			
75	46	3			
Average	72	6			

 $[\]frac{1}{2}$ Based upon 600 sown seed.

Discussion

The need to begin cone collections early arises whenever large numbers of trees must be covered with limited manpower. The true cost of including a portion of immature seed in a collection is not well documented. This case history furnishes a particularly complete documentation because records of seed from each parent could be followed through all extraction, germination, and nursery steps. With 309 parent trees, much of the data can be considered applicable to operational collections. It also provides sound averages and the magnitude of normal variations from the average. The collection year, 1966, was typical of an excellent seed year in all regards with most trees flowering, a good pollination, and generally high seed yields. Lots collected the 10 days before seedfall showed less mold, required minimal effort to process cones and seed, germinated well in nursery beds, and produced excellent plants.

Those lots harvested the first 10 days (before August 23) required several fold efforts in seed extraction and germination, produced very light seed, contained nearly all the lots germinating below 10 percent, suffered heavily from loss after germination, required inordinate efforts to produce enough seedlings for a progeny test, and were the major cause of poor seedbed densities. On a per-seedling basis, at least 10 times the effort was required compared with cone collections made within 10 days of seedfall. Although such comparisons are inexact, they serve to place a better perspective on the true cost of immature seed.

Several alternatives are open to reduce such costs. Seed lots suspected of immaturity may be artificially ripened. Such lots may also be discarded or used only when other seed is exhausted. The obviously better answer is to avoid these problems by providing ample effort to collect only mature cones.

 $[\]frac{2}{}$ Based upon 385 to 4,339 sown seed.

The case history has provided an index to convert the cone cut test to seeds per cone or seeds per bushel. The index should not be used without inspection of the large variation associated with any filled seed count (fig. 2).

Several further observations appear important. Seed averaging below 0.80 gram per 100-seed weight were predominantly from immature lots, and fully ripened lots averaged well above 1.0 gram per 100 seed. Low seed weights thus provide a first clue to immaturity that should become cause to check for other signs such as low germination, bacterial attack of growing points following germination, and low seedbed densities. However, light seed may only indicate small but well-ripened seed.

The inexact correspondence between laboratory and nursery germina-

tion, with nursery germination the higher, was not expected. Much of this variation may have been due to need to stratify fresh seed for the germination test, not considered necessary in this instance. However, the factors involved in the rather large discrepancies in many lots need more investigation.

Of subtle importance is the observation from figures 1 and 3B that seed weight and nursery germination continue to improve steadily for collections made even during the last 2 weeks before seedfall. This suggests that even as seed begins to fall from some trees, on other trees cones are still maturing. The importance may be minor with operational collections. However, with collections intended for genetic testing, such differences in maturity between trees on the same date could lead to nongenetic factors being incorporated into the tests.

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975. Influence of date of cone collection on Douglasfir seed processing and germination: a case history. USDA For. Serv. Res. Pap. PNW-190, 10 p., illus. Pacific Northwest Forest and Range Experiment Station, Portland, Oregon. Cones, seeds, and seedlings from 70 of 309 parent trees were collected too early, resulting in poor cone yields, reduced seed weight, poor germination, bacterial susceptibility, reduced seedbed density, and greater expenditure of time.

KEYWORDS: Cone collection, germination (seed),
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